Influence Factors on Bioreducing U(VI) by *S.*oneidensis

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Abstract. Impact Factors of reducing U(VI) by the *Shewanella* oneidensis(S. oneidensis) were studied in this study. The results showed U(VI) could be reduced effectively at anaerobic environment by S. oneidensis. AQS with concentration of 1.0 mmol/L is an effective accelerator for U(VI) bioreduction. U(VI) initial concentration has the very big influence on reduction of U(VI). Some toxic organic compounds, such as toluenes, trichloroacetic acid and maleic acid, are available to reduce U(VI) efficiently by S. oneidensis and being utilized as electron donors at the same time. Sodium lactate with concentration of 10-15 mmol/L effectively accelerated for U(VI) bioreduction. Metal ions Cu$^{2+}$, Mn$^{2+}$ and Ca$^{2+}$ impact on the reduction of U(VI). equal concentrations of Cu$^{2+}$ and Mn$^{2+}$ could cause varying degrees of inhibition of U(VI) reduction, Ca$^{2+}$ acted as a weak role in promoting the reduction, the coexisting ions of SO$_4^{2-}$ did not markedly influence for reduction efficiency of U(VI).

Introduction

A large number of uranium waste, tailings and acidic radioactive waste water were caused of the uranium mining, especially in situ leaching of uranium. The radionuclides and toxic substances in the uranium mining spread to the groundwater. It seriously threat to human living and ecological environment. Some areas have become sensitive regions. Uranium pollution groundwater contains coexisting ions, radioactive and non-radioactive pollutants. It is difficult to treat by traditional chemical and physical methods for the groundwater next the uranium mining. *S.*oneidensis is a facultative anaerobic organism which exists in anaerobic environment of groundwater or deposition with rich organic matter of uranium mining regions. It can reduce Fe (III), Cr (VI), U(VI) or other heavy metals under anaerobic environment. It has received widespread attention in the aspects of the eco-environmental modification and biological applications. Study on the reduction process and mechanism of U(VI), the removal effect of coexisting ions in water by *S.*oneidensis aren’t comprehensive enough and perfect. The major factors of U(VI) by S. oneidensis were discussed in this study.

Materials and Methods

Strain and Culture Medium

*S.*oneidensis MR-1, Purchased from Marine Culture Collection of China.

Anaerobic culture medium: NaHCO$_3$ 2.5 g/L, NH$_4$Cl 0.25 g/L, KCl 0.1 g/L, NaCl 0.1 g/L, MgSO$_4$·7H$_2$O 0.05 g/L, MgCl$_2$·6H$_2$O 0.2 g/L, KH$_2$PO$_4$ 0.04 g/L, Yeast Extract 1 g/L. A certain amount of sodium lactate was added as electron donors in reduction experiments.

Influence Factors Experiment Methods of the Reduction of U(VI) by *S.* oneidensis

Figure.1 shows the test equipment. The sterilized microbial culture medium with sodium lactate of 10mmol/L, U(VI) of 20mg/L and AQS of 1mmol/L Constant volume to 100mL at pH of 7.0. Purity nitrogen would be bubbled for 15min to remove the oxygen. The bacterial suspension (OD$_{600}$=0.81) was inoculated into the above culture medium under the protection of nitrogen. Finally, seal the bottle mouth and put the bottles into constant temperature shaker (30°C, 120r.min-1). A certain amount of bacteria samples was extracted with a syringe from the sampling tube at regular intervals, in the process nitrogen had been bubbled to ensure anaerobic. The samples would be centrifuged for 10min by 8000r.min-1and the supernatant would be kept for analysis.
Main Reagents and Analysis Testing Method

Main reagents: Uranosouranic oxide (U₃O₈, Analytical reagent), standard uranium solution prepared according to GBW04201; The other reagents were analytically pure.

Results and Discussion

Growth curve of *S. oneidensis*

The growth curves of *S. oneidensis* are shown at 30℃ and 25℃ under the condition of organic medium in Figure 2. As shown in the Figure 2, In the early growth phase, the organisms in the lag phase grew slowly at 30℃, the bacteria quickly grew into logarithmic period during 6-12h, and the biomass reached the maximum at 13h, after this, The total biomass decreased slightly into decline period. Comparing with the experimental group of 30℃, the lag period is longer under the condition of 25℃, and the logarithmic period of bacteria growth is 8-15h, and after 20hrs, the bacteria grew stationary.

![Figure 2. Effect of temperatures on bacteria growth.](image)

The growth curves of *S. oneidensis* are shown in Figure 3 under the condition of inorganic medium at 30℃. The results indicated that the addition of lactate in the experiments stimulates the bacteria growth. To be similar to natural environment for bacteria growth, the inorganic medium with the addition of lactate was employed in the experiments. While the organic medium was adopted in the enrichment culture of bacteria.

Comparison of U(VI) Reduction Effects of Different Redox Systems

Control groups were prepared to accurately verify the contribution of AQS and microorganisms to the experiments. The control groups consisted of bottles with sole 1.0 mmol/L AQS or bacteria. The experimental groups with both bacteria and AQS were performed with AQS concentrations of 1.0 mmol/L. The results are shown in Figure 4. AQS or bacteria reduced U(VI) in the solution. In the control group with only bacteria, approximately 40% of U(VI) was reduced during 4 days. In the group with AQS only, the reduction rate of U(VI) was stable at approximately 30%. Compared with the control groups, the reductive rate of U(VI) of the experimental group with both AQS and bacteria was relatively higher. The reductive removal rate of U(VI) reached 99.0% with AQS of 1.0 mmol/L during 4 days. This rate was more than the superposition of that of the control groups with either sole AQS or bacteria. AQS could be used as electron shuttle vector between U(VI) and electron donor to accelerate U(VI) reduction. AQS of 1.0 mmol/L was added to the subsequent experiments.
Effect of U (VI) Initial Concentration on the U(VI) Reduction by S. oneidensis.

Figure 5 shows the effect of initial concentration of U (VI) on the reduction of U(VI) by S. oneidensis. In the liquid culture with initial U (VI) concentration of 10, 20, 30, 50 mg.L⁻¹ respectively, there were no obvious cell growth within 24h, which may be due to cells’ adaptation process from aerobic bacteria to anaerobic systems, the bacteria in an adjustment period had an increased reduction rate for U (VI) within 48h, U (VI) removal rate increased rapidly with the extension of time, when it came to 96h, U (VI) removal rates up to 68%, 91%, 95%, 90% respectively. However, reduction rate of U (VI) did not change significantly within 48h when the initial U (VI) concentration was 10mg.L⁻¹, but it followed by a rapid reduction, it may be accounted of uranyl phosphate molecule combined by traces H₂PO₄⁻ and U (VI) was restored by the quinone reduction in medium.

Effect of Electron Donor Concentration on the U(VI) Reduction by S. oneidensis

Figure 6 shows the effect of electron donor concentration on the reduction of U(VI) by S. oneidensis in the liquid culture with sodium lactate concentration of 5, 10, 15, 20 mmol.L⁻¹ respectively. The result show electron donor concentration influences on the reduction of U(VI). When the concentration of sodium lactate was 5.0 mmol.L⁻¹ the rate of U(VI) reduction has no obvious increase, When the concentration of sodium lactate increases to 15.0 mmol.L⁻¹ the reduction rate of U (VI) reached 97.34% at 30℃, pH 7.0 and AQS of 1.0 mmol/L for 7 days. It may be due to U(VI) efficient reduction by S. oneidensis with enough electron donor, in addition, it may exists the balanced relationship between the electron donor and acceptor.

Effect of Toxic Organic Compounds on the U (VI) Reduction by S. oneidensis

In order to investigate the toxic organic compounds in the environment such as toluene, trichloroacetic acid, maleic acid on the U (VI) reduction, the experiments were carried out at the concentrations of organic compounds of 10mmol.L⁻¹, The effects of toxic organic compounds on U(VI) reduction are plotted in Figure 7. As seen from the figure7, the organics can accelerate the reduction of U(VI). The experimental group with the addition of toluene, trichloroacetic acid and maleic acid respectively, the removal rate of U(VI) was 97.20%, 96.64%, 97.91% respectively, during 5 days of reduction by microorganisms. Compared with the control group without toxic organics, this removal rate of U(VI) increased by 24.25%, 23.69%, 24.96% respectively. It may be S. oneidensis could utilize toxic organic compounds as electron donor and promote the U(VI) reduction.
Effect on Coexisting Metal Ions of Cu$^{2+}$, Mn$^{2+}$, Ca$^{2+}$ on Reduction of U (VI)

Biological reduction of U(VI) are often affected by the coexistence ions in water. Figure 8 shows the effect of Cu$^{2+}$, Mn$^{2+}$ and Ca$^{2+}$ on U(VI) reduction. Putting 2.0 mmol.L$^{-1}$ of Cu$^{2+}$, Mn$^{2+}$, Ca$^{2+}$ into the experimental group, The results showed U(VI) reduction rate were 3.72%, 31.78%, 88.88% respectively for 48h, and 82.40% of removal rate without adding metal ion for 48h. Cu$^{2+}$, Mn$^{2+}$ can significantly inhibit U(VI) reduction, and the effect of Cu$^{2+}$ is greater than Mn$^{2+}$; Ca$^{2+}$ acted as a weak role in promoting the reduction of U(VI). Cu$^{2+}$ can caused the inhibition of reduction of U(VI) for the dehydrogenase protein from respiratory chain losing ability of oxidation electron donor with the combination cu$^{2+}$and the active center of the protein.

Conclusions
(1) The initial U(VI) concentration has obvious influence on reduction of U(VI) and the optimum U(VI) concentration is 30mg/L with removal rate of 95.02% for 4 days.
(2) AQS as electron shuttle carrier can stimulate the reduction of U(VI). The concentration of sodium lactate has some influence on the reduction of U (VI), and the removal rate reached 97.34% with sodium lactate of 15mol.L at 30°C, pH 7.0 and AQS of 1.0 mmol/L for 7 days.
(3) S. oneidensis can use the toxic organics in the environment as donor and accelerate the reduction of U(VI).
(4) Concentrations of Cu$^{2+}$ and Mn$^{2+}$ could cause varying degrees of inhibition of U(VI) reduction, Ca$^{2+}$ acted as a weak role in promoting the reduction.

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References